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Impact of HIV status and vaccination schedule on bacterial nasopharyngeal carriage following infant immunisation with the pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine in South Africa

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ABSTRACT

Background: Nasopharyngeal carriage (NPC) of *Streptococcus pneumoniae* is a precondition for pneumococcal disease and a source of transmission. This trial evaluated NPC of *S. pneumoniae* and other pathogens post-vaccination with the pneumococcal non-typeable *Haemophilus influenzae* (NTHi) protein D conjugate vaccine (PHiD-CV) in human immunodeficiency virus (HIV)-infected (HIV+), HIV-exposed-uninfected (HEU), and HIV-unexposed-uninfected (HUU) South African children.

Methods: In this phase III, open, single-centre, controlled study (ClinicalTrials.gov: NCT00829010), 484 children were stratified by HIV status: 83 HIV+, 101 HEU, and 300 HUU. HIV+ and HEU children received a 3 + 1 PHiD-CV vaccination schedule: primary vaccination, age 6/10/14 weeks, and booster dose, age 9–10 months. HUU infants were randomised (1:1:1) to 3-dose priming and booster (HUU/3+1); 3-dose priming without booster (HUU/3+0); or 2-dose priming and booster (HUU/2+1). Bacterial NPC was assessed 8 times up to 24–27 months of age.

Results: Overall pneumococcal carriage rates were similar across 3+1 groups irrespective of HIV status; trends towards higher carriage rates in the HIV+ than HEU and HUU/3+1 groups were observed at 24–27 months of age. In HUU children, carriage of any pneumococcal serotype was similar for the three different dosing schedules at all timepoints; carriage of vaccine-type pneumococci tended to be lower at 16–19 months and 24–27 months of age in children who had received a booster dose (HUU/2+1 and HUU/3+1 groups) than in the HUU/3+0 group. Carriage rates of NTHi, *Staphylococcus aureus* and *Moraxella catarrhalis* were comparable between all groups.

Conclusions: HIV infection or exposure did not seem to alter the effect of PHiD-CV on pneumococcal NPC in children during their first 2 years of life. NPC prevalence of vaccine-type pneumococci following vaccination series tended to be lower in children who had received a booster dose in comparison to those who had not.

Abbreviations: HEU, HIV-exposed-uninfected; HIV, human immunodeficiency virus; HIV+, HIV-infected; HUU, HIV-unexposed-uninfected; IPD, invasive pneumococcal disease; NPC, nasopharyngeal carriage; NTHi, non-typeable *Haemophilus influenzae*; NVT, non-vaccine and non-vaccine-related type; PCR, polymerase chain reaction; PCV, pneumococcal conjugate vaccine; PCV7, 7-valent pneumococcal conjugate vaccine; PHiD-CV, pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine; VT, vaccine-type.

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1. Introduction

Streptococcus pneumoniae plays an important role in invasive bacterial disease, upper respiratory tract infections, and pneumonia [1]. The World Health Organization recommends immunisation with a pneumococcal conjugate vaccine (PCV) for young children worldwide [2]. Nasopharyngeal colonisation by *S. pneumoniae* is a precondition for developing invasive pneumococcal disease (IPD) [3]. Impact of PCV vaccination on nasopharyngeal carriage (NPC) provides an indication of the vaccine's biological activity and likelihood to reduce transmission [4], which in consequence may provide protection against IPD in unvaccinated people as an indirect effect (herd protection) [5].

The pneumococcal non-typeable *Haemophilus influenzae* (NTHi) protein D conjugate vaccine (PHiD-CV) has shown efficacy against NPC of vaccine-type (VT) pneumococci [6–10]. This was seen with both 2 + 1 (2 primary doses plus booster) and 3 + 1 (3 primary doses plus booster) PHiD-CV schedules; in a study assessing both schedules, vaccine efficacy against VT carriage was 30% [3 + 1] and 23% [2 + 1] 6 months post-primary vaccination and 40% [3 + 1] and 38% [2 + 1] 3 months post-booster vaccination in Finnish children enrolled between 6 weeks and 6 months of age [9]. In this large efficacy study, PHiD-CV also induced a significant reduction in vaccine-related serotype 19A NPC when administered according to the 3 + 1 schedule [9].

NTHi is another pathogen associated with respiratory diseases. The use of NTHi-derived protein D as carrier for pneumococcal polysaccharides in PHiD-CV may afford protection against this pathogen. Transient vaccine efficacy against NTHi NPC following PHiD-CV vaccination has been observed in some studies but not in others [6,8–10].

Reducing the number of PCV primary-series doses with a subsequent booster dose (i.e. 2 + 1 vs 3 + 1 schedule) has been shown to maintain the vaccine effect on pneumococcal NPC [11,12], and to maintain protection against IPD in high-income countries [13–15], but data from developing countries and in human immunodeficiency virus (HIV)-infected (HIV+) or HIV-exposed children are still lacking.

Our study was conducted in Soweto, South Africa, where PCV vaccination according to a 2 + 1 schedule at the age of 6 weeks, 14 weeks and 9 months was introduced in the national immunisation programme in April 2009 (7-valent PCV [PCV7] transitioned to 13-valent PCV in May 2011) [16,17]. We performed a study assessing the safety and immunogenicity of PHiD-CV in HIV+, HIV-exposed-uninfected (HEU) and HIV-unexposed-uninfected children (HUU). Different vaccination schedules were assessed in HUU children. In addition, NPC was also assessed as a biological effect of the vaccine that could potentially have an impact on transmission and result in herd effects which are important for disease prevention. We previously reported the immunogenicity and safety of PHiD-CV (3 + 1 schedule) in HIV+, HEU, and HUU children [18]. Additionally, PHiD-CV immunogenicity and safety, when administered according to different vaccination schedules (3 + 1, 3 + 0, 2 + 1) in HUU children were also reported [19]. Here we report the results of the assessment of NPC in all study groups.

2. Material and methods

2.1. Study design and participants

This was a phase III, open, controlled, single-centre, partially randomised study conducted in South Africa between February 2009 and June 2012. Study participants were infants 6–10 weeks of age at first vaccination, without any known or suspected health problems (other than HIV infection or exposure) that would contraindicate initiation of routine immunisations. Children were stratified according to their HIV status: HIV+ (HIV-positive infants born to HIV-positive mothers), HEU (HIV-negative infants born to HIV-positive mothers), and HUU (HIV-negative infants born to HIV-negative mothers). Inclusion/exclusion criteria and HIV assessments (**Text S1**) were detailed previously together with the results of the primary and part of the secondary objectives [18,19]. Here, we present evaluation of bacterial NPC according to the HIV status and vaccination schedules.

The study was conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki, and with the approval of an independent ethics committee (Wits Human Research Ethics Committee). Written informed consent was obtained from the parent(s) or legally acceptable representative (s) of each child before any study-specific procedure. This study was registered at www.clinicaltrials.gov (NCT00829010). A protocol summary is available at www.gsk-clinicalstudyregister.com (study ID: 111634).

2.2. Treatment allocation and study vaccines

The study included 5 parallel groups: HIV+ and HEU infants received a 3 + 1 PHiD-CV (*Synflorix*, GSK, Belgium) vaccination schedule, while HUU infants were randomised (1:1:1) into 3 groups to receive different PHiD-CV vaccination schedules: a 3 + 1 (HUU/3+1), 3 + 0 (HUU/3+0), or 2 + 1 schedule (HUU/2+1), at the ages presented in Fig. 1. PHiD-CV was administered intramuscularly in the right anterolateral thigh. Compositions of PHiD-CV and routinely co-administered vaccines, including diphtheria-tetanus-whole cell pertussis-hepatitis B vaccine combined with lyophilised *H. influenzae* type b tetanus conjugate vaccine, human rotavirus vaccine, measles vaccine, and oral polio vaccine have been described previously [18,19].

2.3. Microbiological assessment

Dacron-tipped nasopharyngeal swabs (Cat# 151D, MedicalWire Equipment Co. Ltd.; Wiltshire) were collected at 8 timepoints from each participant up to 24–27 months of age (Fig. 1), placed in skim milk-tryptone-glucose-glycerol transport medium, and stored at −70 °C until testing and identification of pathogens using routine microbiological methods and/or polymerase chain reaction (PCR) (**Text S2**). Viable pneumococci were serotyped by the Quellung reaction using specific antisera (Statens Serum Institute, Copenhagen, Denmark), and slide agglutination and PCR were used for serotyping and confirmation of *H. influenzae*. Pneumococcal serotypes 6C and 6D were differentiated from serotype 6A and 6B by the Quellung reaction.

2.4. Statistical analysis

Sample size considerations were previously reported [18,19]. The carriage analysis was descriptive and based on the total vaccinated cohort, including all children with ≥1 administered vaccine dose. The occurrence of pathogens/serotypes in the nasopharynx was evaluated at each swabbing timepoint, as the percentage of children in each group with positive results for each pathogen/serotype. The occurrence across post-vaccination visits is the percentage of children with positive swabs associated to the specified bacteria after at least one visit (excluding the pre-vaccination visit – Visit 1). Co-colonisation was defined as the number of children with at least one swab positive for both considered pathogens divided by the number of children with swab results. Cumulative

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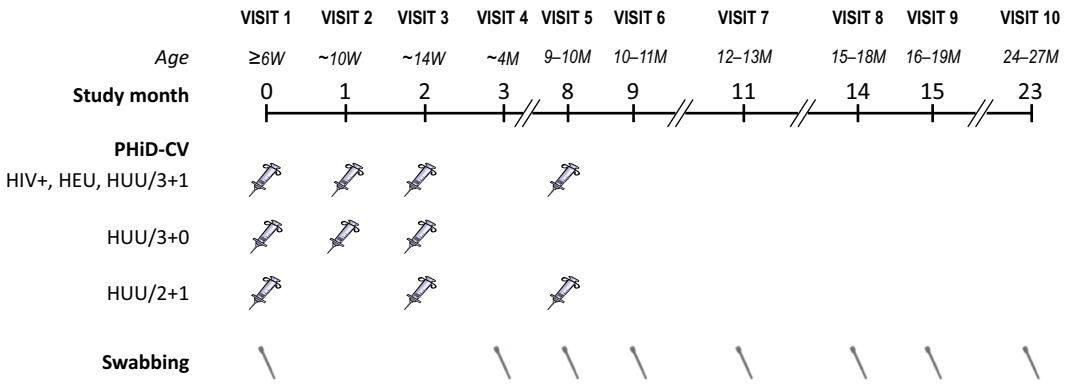

Fig. 1. Study design. PHiD-CV, pneumococcal non-typeable *H. influenzae* protein D conjugate vaccine; W, weeks; M, months; HIV+, HIV-infected children; HEU, HIV-exposed-uninfected children; HUU, HIV-unexposed-uninfected children; syringe depicts PHiD-CV vaccination (other paediatric vaccines were co-administered per national immunisation programme).

acquisition was defined as the occurrence of bacterial pathogens/serotypes not detected at any of the previous sampling timepoints for which a result was available. Once identified in a child, a new pathogen/serotype contributed to positive results, even if at subsequent timepoints the child did not carry it anymore or acquired a new pathogen/serotype. Statistical analyses were performed using the Statistical Analysis System (SAS) Drug and Development (SDD) web portal version 3.5 and SAS version 9.2.

3. Results

3.1. Study participants

A total of 484 infants were enrolled (HIV+: 83, HEU: 101, HUU: 300; Fig. 2). Four infants initially considered HIV+ were reallocated to HEU, because they were born to an HIV+ mother, confirmed as being HIV+ by HIV deoxyribonucleic acid PCR at screening, but had undetectable HIV ribonucleic acid at visit 1 (3 infants) or undetermined HIV status at retesting (1 infant). One infant, who was wrongly randomised in the HUU/3+1 group, was considered HEU for the analyses. Demographic characteristics were comparable between groups in terms of age, ethnicity and gender (**Table S1**). All HIV+ infants were on antiretroviral therapy by 9–10 months of age. Overall compliances for collecting nasopharyngeal swabs (>90%) and culture of swabs (≥99.8% per visit) were high.

3.2. NPC according to HIV status in children receiving the 3 + 1 schedule

3.2.1. S. pneumoniae

Overall pneumococcal NPC increased over time in all groups, with the major increases observed by 4 months of age. Colonisation prevalence with any serotype ranged between 23.8% and 27.7% pre-vaccination, 58.0–63.3% 1 month post-primary vaccination, 63.3–66.7% 1 month post-booster, and 64.1–79.5% at 24–27 months of age (Fig. 3**A**, **Table S2**). No differences between groups were detected, except a tendency towards higher NPC rates in HIV+ children at study end. The most prevalent pneumococcal serotypes across all visits and across 3+1 groups were serotypes 6B, 6A, 19F, and 23F.

NPC rates of VT pneumococci remained within the same ranges across all visits and were comparable between groups. There was a trend (although not statistically significant) for higher VT NPC in the HIV+ group at most visits. NPC of VT pneumococci ranged between 12.9% and 15.7% at pre-vaccination, 25.5–30.9% 1 month post-primary vaccination, 15.6–32.5% 1 month post-booster, and 16.3–26.0% at 24–27 months of age (Fig. 3**B**, **Table S2**). For vaccine-related serotypes 6A and 19A, NPC rates were in similar ranges across groups at most timepoints (**Table S2**).

Occurrence of pneumococcal non-vaccine and non-vaccine-related types (NVTs) in nasopharyngeal swabs increased over time in all groups. Colonisation with NVT pneumococci ranged between 4.0% and 8.4% at pre-vaccination, 22.2–25.5% 1 month post-primary vaccination, 22.1–30.6% 1 month post-booster, and 28.3–38.4% at the last visit (Fig. 3**C**, **Table S2**). Similar NPC rates of NVTs were observed in all groups, except a tendency towards higher rates in HIV+ children at study end.

3.2.2. Other pathogens

NTHi colonisation increased over time, with similar rates in all groups, ranging between 11.9% and 17.2% at pre-vaccination, and 45.7–53.6% at the last visit (Fig. 4**A**, **Table S3**). Colonisation with *Staphylococcus aureus* decreased over time, with similar rates across groups. The highest colonisation rate with *S. aureus* was 56.0% at pre-vaccination while the lowest was 9.2% at 15–18 months of age (HUU/3+1 group) (Fig. 4**B**, **Table S3**). NPC rates of *Moraxella catarrhalis*, detected by PCR, increased over time in all groups, with a tendency towards lower rates in HIV+ children prior to the booster dose (Fig. 4**C**, **Table S3**). The highest carriage rates were 87.7% and 90.7% (HIV+ and HUU/3+1 groups) at 24–27 months of age, and 94.7% at 16–19 months of age (HEU group). PCR results for *Streptococcus pyogenes* and *Pseudomonas aeruginosa* are presented in **Table S4**.

3.2.3. Co-colonisation rates

The percentages of children in the 3+1 groups co-colonised at least once across visits with two different *S. pneumoniae* serotypes ranged between 14.5% and 19.8% for any serotype and between 1.0% and 3.0% for two different VTs. The percentages of children co-colonised at least once across visits with two different pathogens ranged between 76.0% and 80.7% for *S. pneumoniae* and NTHi, 47.0–56.6% for *S. pneumoniae* and *S. aureus,* and 95.0–96.0% for *S. pneumoniae* and *M. catarrhalis* in all 3+1 groups.

3.2.4. Cumulative acquisition rates of S. pneumoniae

Cumulative acquisition ranged from 43.2–48.0% to 96.7–98.6% for any pneumococcal serotype (**Fig. S1A**), from 14.3–22.4% to 54.4–62.9% for VT (**Fig. S1B**), from 1.0–5.1% to 24.7–32.2% for serotype 6A, from 0.0–3.1% to 15.5–17.8% for serotype 19A and from 19.4–21.0% to 74.2–76.7% for NVT (**Fig. S1C**) from 1 month post-primary vaccination to 24–27 months of age.

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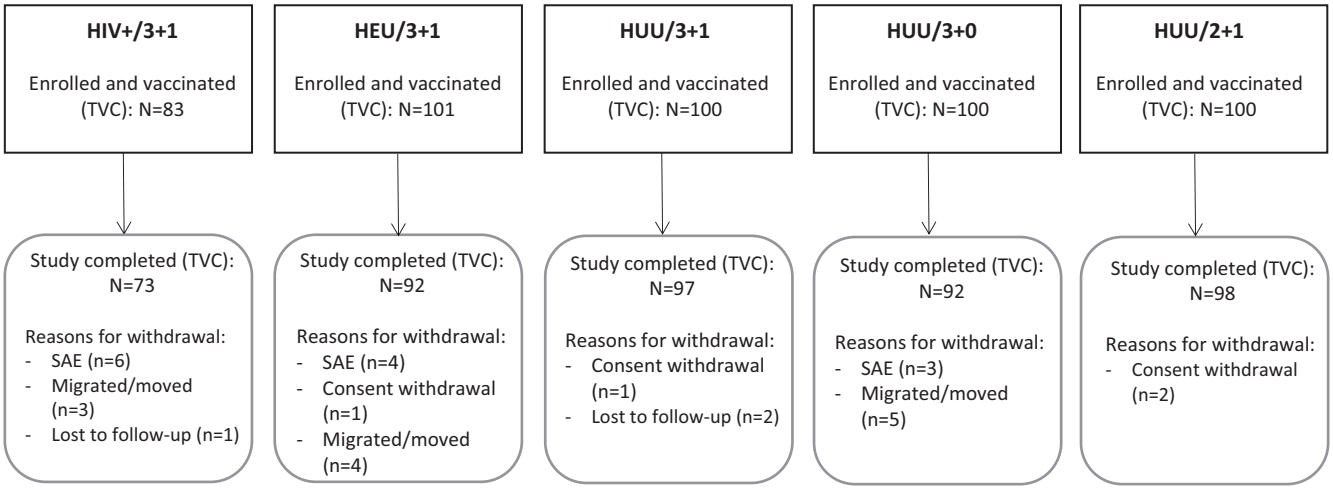

Fig. 2. Flow diagram of study participants. TVC, total vaccinated cohort; N, number of infants per group; n, number of infants with the specified characteristic; SAE, serious adverse event; HIV+, HIV-infected children; HEU, HIV exposed-uninfected children; HUU, HIV-unexposed-uninfected children; 3+1, 3 primary doses of pneumococcal non-typeable *H. influenzae* protein D conjugate vaccine (PHiD-CV) at 6, 10, 14 weeks and a booster dose of PHiD-CV at 9–10 months of age; 3+0, 3 primary doses of PHiD-CV at 6, 10, 14 weeks of age without booster dose; 2+1, 2 primary doses of PHiD-CV at 6 and 14 weeks and a booster dose of PHiD-CV at 9–10 months of age.

3.3. NPC according to vaccination schedule in HUU children

3.3.1. S. pneumoniae

Overall pneumococcal NPC increased over time in all groups, with the major increases observed by 4 months of age and rates in similar ranges across schedules at each timepoint. Colonisation prevalence with any serotype ranged between 17.0% and 30.0% at pre-vaccination, and 64.9–67.3% at 24–27 months of age (Fig. 5**A**, **Table S2**). The most prevalent serotypes across visits were 6B, 6A, 23F and 19F.

At 1 month post-primary vaccination, there was a trend towards lower VT colonisation rates in children receiving 3 primary doses compared to those receiving 2 primary doses, which disappeared by 9–10 months of age. VT NPC tended to be higher at 16–19 months and 24–27 months of age in children not receiving a booster dose (3 + 0 schedule) (Fig. 5**B**, **Table S2**). NPC rates of vaccine-related serotypes 6A and 19A were in similar ranges for the different vaccination schedules at all timepoints (**Table S2**).

Colonisation with NVTs increased up to 9–10 months of life and remained stable thereafter, ranging between 5.0% and 12.0% at pre-vaccination, 24.2–30.6% 1 month post-primary vaccination, 30.6–36.7% at 10–11 months of age, and 28.3–30.9% at last visit (Fig. 5**C**, **Table S2**).

3.3.2. Other pathogens

Occurrences of NTHi, *S. aureus,* or *M. catarrhalis* in nasopharyngeal swabs were similar for the different vaccination schedules at all timepoints. NPC rates of NTHi increased over time, ranging between 11.2% and 17.2% at pre-vaccination and 53.1–55.4% at 24–27 months of age (Fig. 6**A**, **Table S3**). NPC rates of *S. aureus* decreased over time, with the highest colonisation prevalence measured at pre-vaccination for the HUU/3+0 group (57.0%) and the lowest at 15–18 months of age for the HUU/3+1 group (9.2%) (Fig. 6**B**, **Table S3**). Colonisation with *M. catarrhalis* increased over time, with the major increases observed by 4 months of life. Across groups, NPC rates of *M. catarrhalis* ranged between 39.0% and 44.0% at pre-vaccination, and the highest rates were 90.7% (HUU/3+1 group) and 93.9% (HUU/2+1 group) at 24–27 months of age, and 91.5% at 15–18 months of age (HUU/3+0 group) (Fig. 6**C**, **Table S3**). PCR results for *S. pyogenes* and *P. aeruginosa* are presented in **Table S4**.

3.3.3. Co-colonisation rates

The percentages of HUU children co-colonised at least once across visits with two different pneumococcal serotypes ranged between 16.0% and 18.0% for any serotype and between 2.0% and 3.0% for two different VTs. The percentages of children co-colonised at least once across visits with two different pathogens ranged between 76.0% and 81.0% for *S. pneumoniae* and NTHi, 47.0–59.0% for *S. pneumoniae* and *S. aureus*, and 93.0–97.0% for *S. pneumoniae* and *M. catarrhalis* in all HUU groups.

3.3.4. Cumulative acquisition rates of S. pneumoniae

Cumulative acquisition rates ranged from 43.2–57.1% to 97.9–99.0% for any *S. pneumoniae* serotype (**Fig. S2A**), from 13.7–26.5% to 62.9–66.3% for VT (**Fig. S2B**), from 0.0–6.3% to 19.6–29.3% for serotype 6A, from 1.1–3.1% to 15.5–21.7% for serotype 19A and from 19.4–28.6% to 74.2–80.4% for NVT (**Fig. S2C**) from 1 month post-primary vaccination to 24–27 months of age.

4. Discussion

Our study suggests that in infants and young children, HIV status does not alter the effect of PHiD-CV vaccination on pneumococcal NPC, when administered in a 3 + 1 schedule. *S. pneumoniae* NPC appeared comparable between HEU and HUU children both post-primary and post-booster vaccination. Colonisation with pneumococci was also in the same range in the HIV+, but tended to be higher at 24–27 months of age (any, VT and NVT carriage) than in the HEU and HUU groups. In the same trial, we found that anti-pneumococcal antibody concentrations were similar in HIV+, HEU and HUU children for most VTs post-primary vaccination and post-booster [18]. However, it is important to mention that all HIV+ children had World Health Organization clinical stage 1 at enrolment [20], and only few progressed to a higher stage, as all HIV+ participants received antiretroviral therapy by the age of 9–10 months. Thus, extrapolation of these results to children with moderately or severely symptomatic HIV should be done with caution. We also assessed NPC of other bacterial pathogens; no major differences between HIV+, HEU and HUU children (3 + 1 schedule) were observed.

In a previous study with PCV7, VT NPC was also similar in HIV+ and HIV-uninfected South African children. However, the prevalence of non-PCV7 type pneumococci, *S. aureus* (pre-PCV7-

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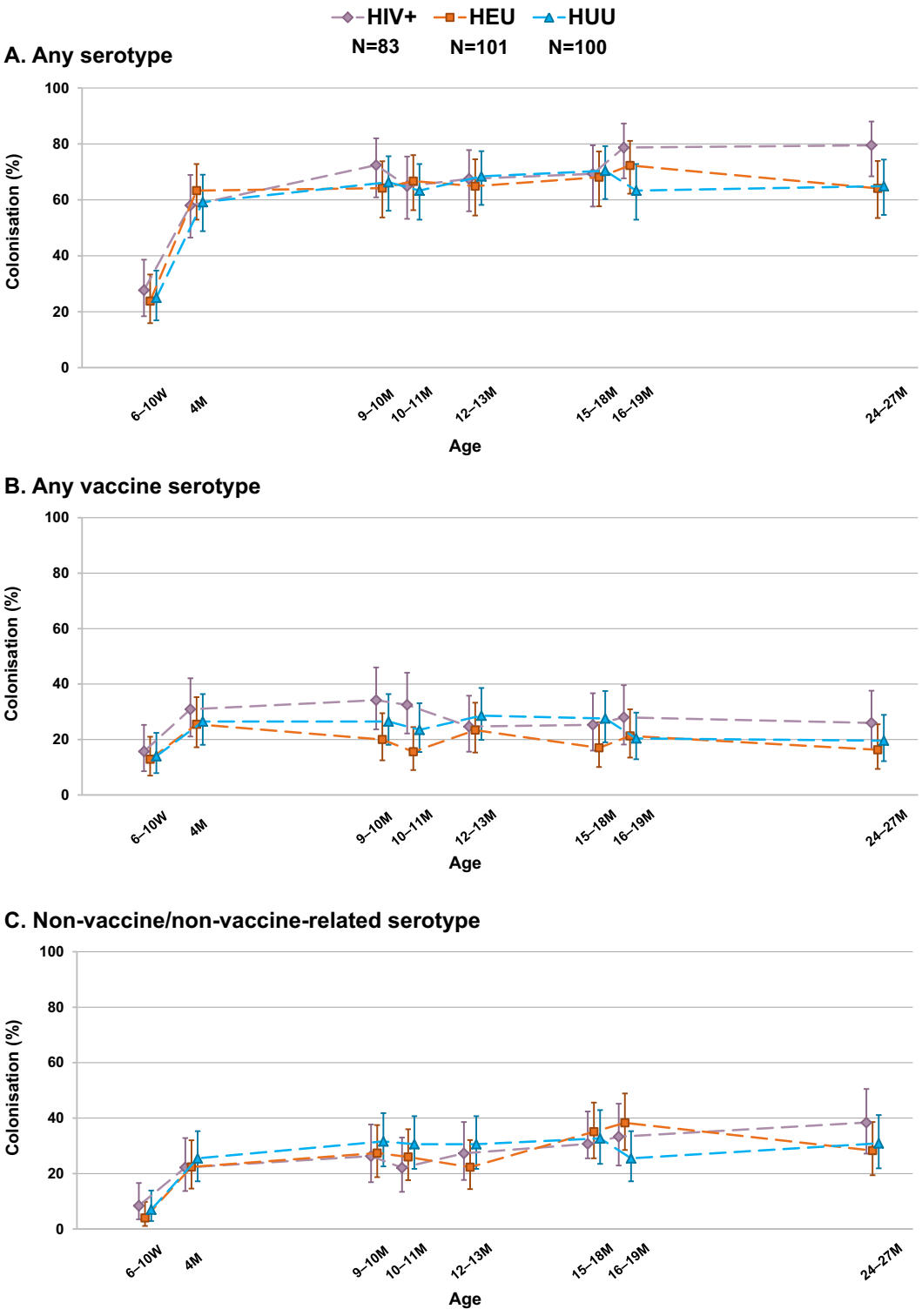

Fig. 3. Nasopharyngeal carriage of *S. pneumoniae* in 3+1 groups with different HIV status (total vaccinated cohort). N, number of infants included in each group; W, weeks; M, months; HIV+, HIV-infected infants; HEU, HIV-exposed-uninfected infants; HUU, HIV unexposed-uninfected infants; 3+1, 3 primary doses of pneumococcal non-typeable *H. influenzae* protein D conjugate vaccine (PHiD-CV) at 6 W, 10 W, 14 W and a booster dose of PHiD-CV at 9–10 M of age. Error bars depict 95% confidence intervals. Non vaccine/non-vaccine related serotype is any serotype not included in the vaccine and not belonging to the same serogroup as a vaccine serotype.

vaccination), and *H. influenzae* (post-PCV7-vaccination) colonisation was lower in HIV+ than HIV-uninfected children [21]. The differences compared to our study should be evaluated with caution considering the differences in settings and design of the studies, and the external factors that can influence bacterial NPC [22].

Additionally, we evaluated the impact of different PHiD-CV vaccination schedules in HUU children and observed a trend towards lower VT NPC rates at 16–19 months and 24–27 months of age in the HUU/3+1 and HUU/2+1 groups versus the HUU/3+0 group (post-booster timepoints). This trend coincides with higher anti-

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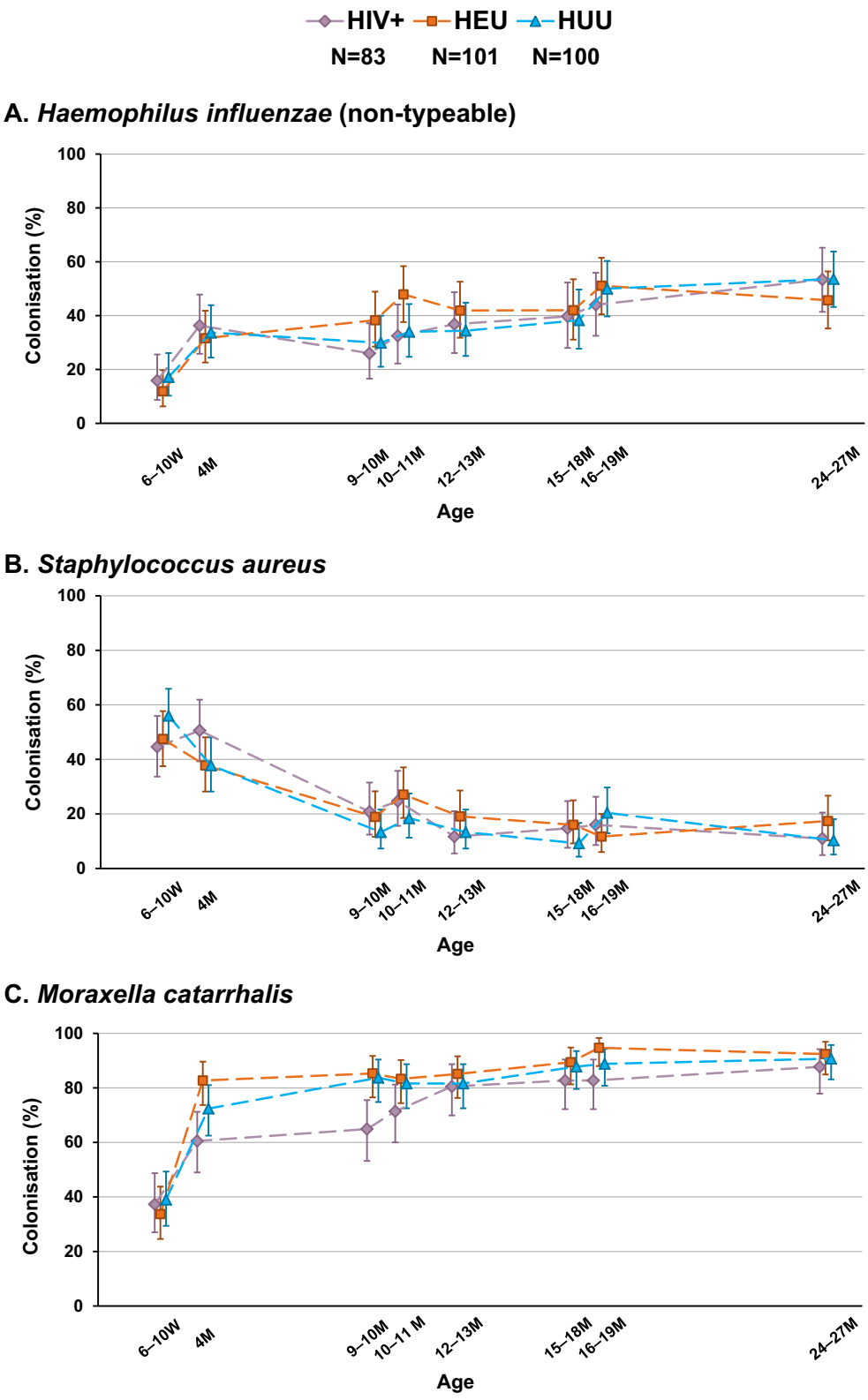

Fig. 4. Nasopharyngeal carriage of other bacterial pathogens in 3+1 groups with different HIV status (total vaccinated cohort). N, number of infants included in each group; W, weeks; M, months; HIV+, HIV-infected infants; HEU, HIV-exposed-uninfected infants; HUU, HIV unexposed-uninfected infants; 3+1, 3 primary doses of pneumococcal non-typeable *H. influenzae* protein D conjugate vaccine (PHiD-CV) at 6 W, 10 W, 14 W and a booster dose of PHiD-CV at 9–10 M of age. Error bars depict 95% confidence intervals. Note: Non-typeable *H. influenzae* was identified and serotyped by routine microbiological methods and polymerase chain reaction, and further confirmed by polymerase chain reaction; *S. aureus* was identified by culture-based methods; *M. catarrhalis* was identified by polymerase chain reaction.

body geometric mean concentrations and opsonophagocytic activity geometric mean titres elicited by the booster dose administered in the HUU/3+1 and HUU/2+1 groups at 9–10 months of age, compared with those observed in children who did not receive a booster (3+0 group) [19]. No differences in NPC of other pathogens were observed between the various vaccination schedules.

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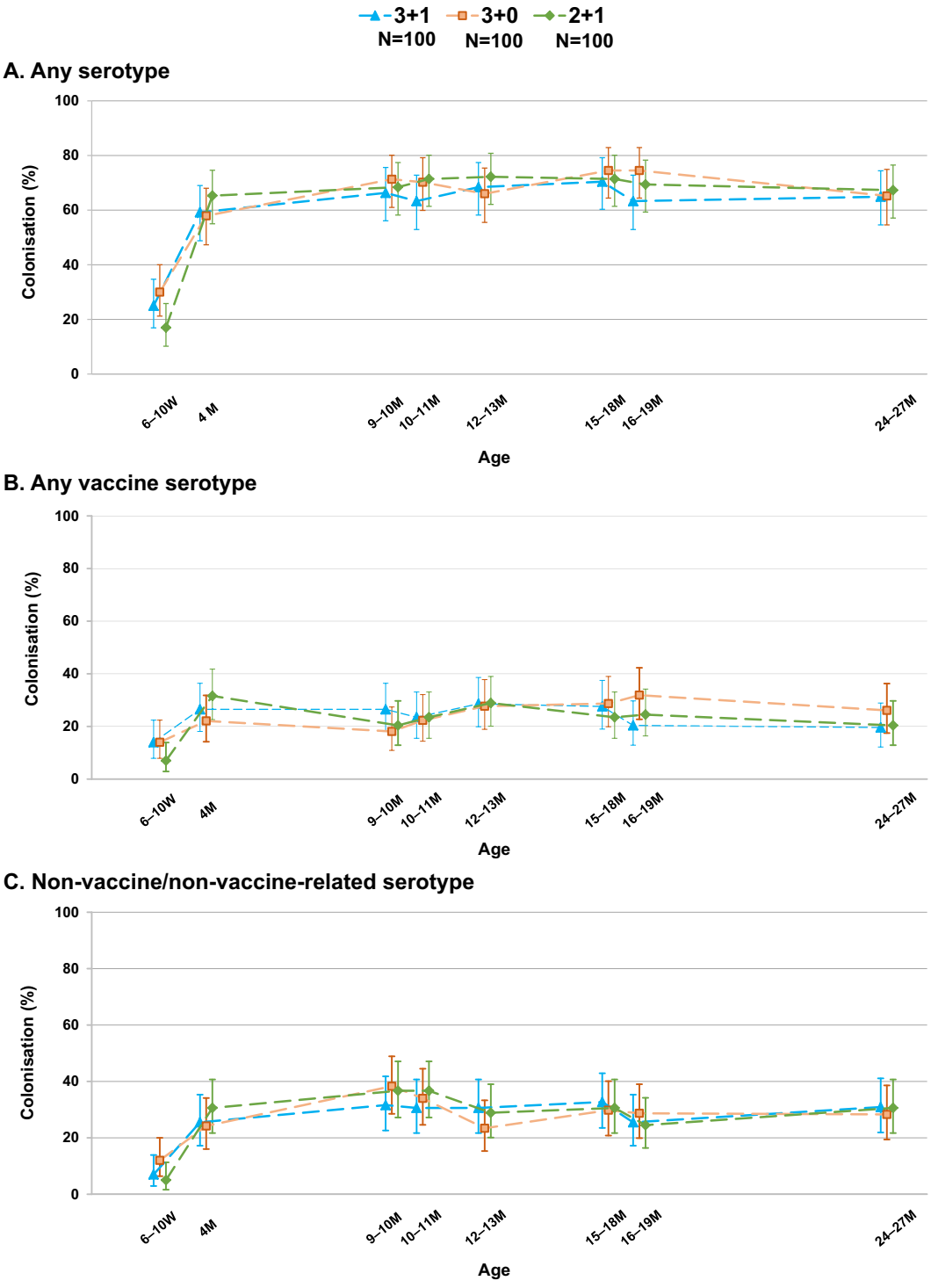

Fig. 5. Nasopharyngeal carriage of *S. pneumoniae* in HUU groups with different vaccination schedules (total vaccinated cohort). N, number of infants included in each group; W, weeks; M, months; HUU, HIV-unexposed-uninfected infants; 3+1, 3 primary doses of pneumococcal non typeable *H. influenzae* protein D conjugate vaccine (PHiD-CV) at 6 W, 10 W, 14 W and booster dose of PHiD-CV at 9–10 M of age; 3+0, 3 primary doses of PHiD-CV at 6 W, 10 W, 14 W of age without booster dose; 2+1, 2 primary doses of PHiD-CV at 6 W, 14 W and a booster dose of PHiD-CV at 9–10 M of age. Error bars depict 95% confidence intervals. Non-vaccine/non vaccine-related serotype is any serotype not included in the vaccine and not belonging to the same serogroup as a vaccine serotype.

A previous study conducted in South Africa has shown that PCV7, when administered according to the recommended 2 + 1 schedule, reduced the risk of colonisation with VTs in vaccinated versus unvaccinated children [23]. Moreover, VT colonisation and acquisition rates were similar to a historical cohort vaccinated with a 3 + 1 schedule, suggesting that similar indirect protection against IPD could be derived from either schedule [23]. Another PHiD-CV trial conducted in Finland reported similar ranges of pneumococcal colonisation rates (any serotype or VT) after 2 + 1 or 3 + 1 vaccination schedules with booster doses administered at 11–12 months of age, except for the last timepoint (18–22 months of age), when the reduction in NPC compared to the control group was greater

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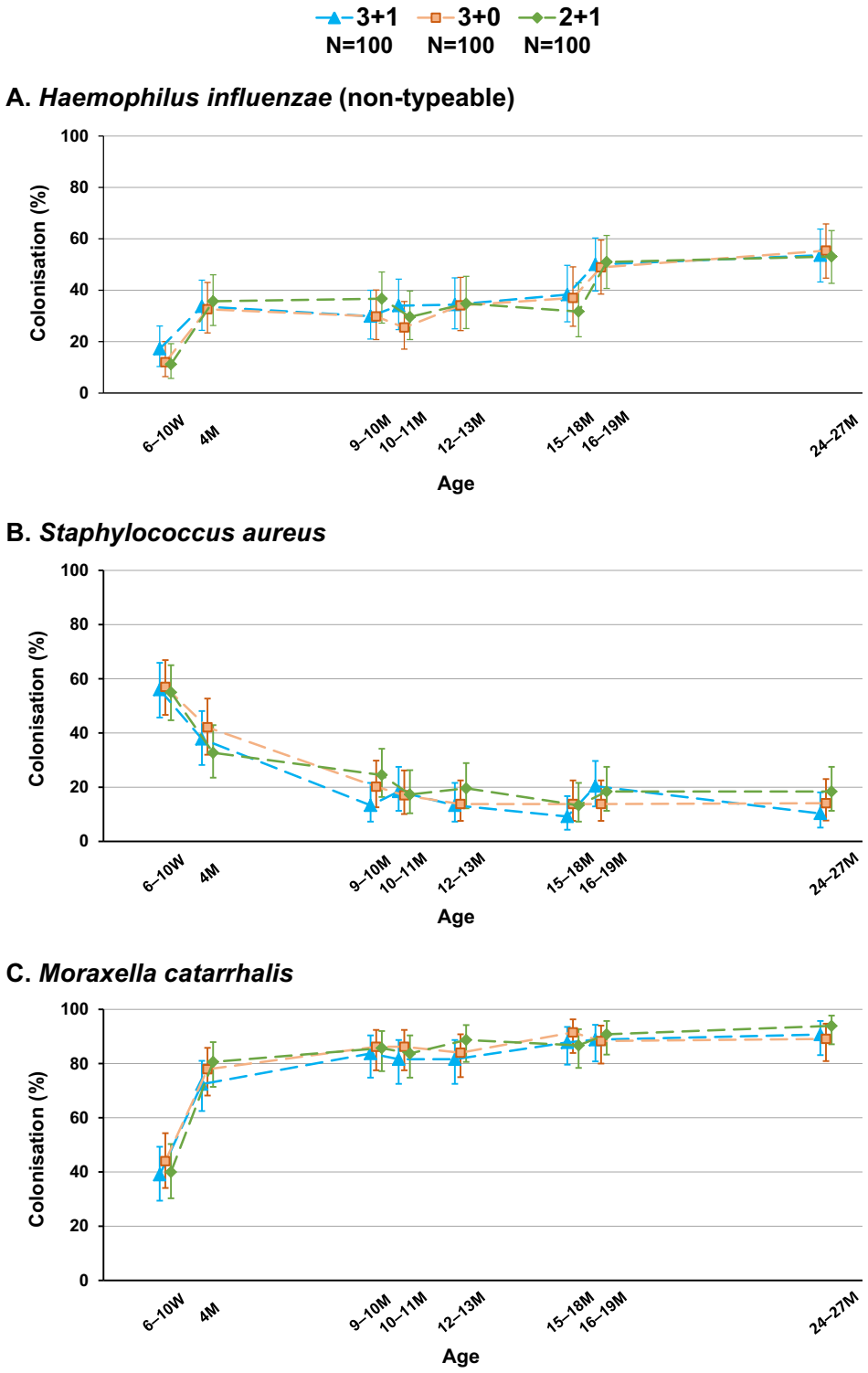

Fig. 6. Nasopharyngeal carriage of other bacterial pathogens in HUU groups with different vaccination schedules (total vaccinated cohort). N, number of infants included in each group; W, weeks; M, months; HUU, HIV-unexposed-uninfected infants; 3+1, 3 primary doses of pneumococcal non typeable *H. influenzae* protein D conjugate vaccine (PHiD-CV) at 6 W, 10 W, 14 W and booster dose of PHiD-CV at 9–10 M of age; 3+0, 3 primary doses of PHiD-CV at 6 W, 10 W, 14 W of age without booster dose; 2+1, 2 primary doses of PHiD-CV at 6 W, 14 W and a booster dose of PHiD-CV at 9–10 M of age. Error bars depict 95% confidence intervals. Note: Non-typeable *H. influenzae* was identified and serotyped by routine microbiological methods and polymerase chain reaction, and further confirmed by polymerase chain reaction; *S. aureus* was identified by culture-based methods; *M. catarrhalis* was identified by polymerase chain reaction.

with the 3 + 1 schedule [9]. In a recent trial in The Gambia assessing NPC in children after 3 + 0 and 2 + 1 (booster dose administered at 9 months of age) PHiD-CV vaccination schedules, acquisition of pneumococcal NPC was similar across post-vaccination visits, but prevalence seemed higher (82–91%) than in our study (<80%) [12]. Impact of PHiD-CV vaccination schedules on NPC should be interpreted with caution considering that in European and South American settings [7–10], pneumococcal NPC rates were generally

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Fig. 7. Plain Language Summary.

lower than those observed in our and other African studies [12,23–25]. Similarly to our study, the phase II trial performed in The Gambia took place at the start of PCV implementation in national immunization program [12], which might impact the serotype distribution and influence the dynamics of VT transmission [26].

NPC is increasingly being advocated as an additional measure of potential efficacy of pneumococcal vaccines [4]. Reduced carriage of *S. pneumoniae* decreases transmission and in consequence exposure of unvaccinated individuals, resulting in substantial indirect effects. In a systematic review of studies predominantly from high-income countries however, only a 3 + 1 schedule showed significant indirect effects on VT NPC, but all schedules considered (2 + 1, 3 + 0, and 3 + 1) demonstrated indirect effects on VT IPD [27].

Our study was limited by the sample size and the fact that the evaluation of colonisation was a secondary objective. All comparisons were descriptive and the study was not sufficiently powered to assess differences, thus confidence intervals are large and results should be interpreted with caution. Another limitation was the absence of an unvaccinated control group because the study was conducted in an area with high risk for pneumococcal disease and the standard of care in South Africa at the time of the study became PCV7.

A plain language summary contextualizing the results and potential clinical research relevance and impact is displayed in Fig. 7.

5. Conclusions

HIV infection or exposure did not seem to alter the effect of PHiD-CV vaccination on the prevalence of nasopharyngeal bacterial carriage and cumulative acquisition of *S. pneumoniae* in children during their first 2 years of life. When assessing different infant vaccination schedules in HUU children, a trend towards lower VT colonisation was observed at 16–19 months and 24–27 months of age in children receiving a booster dose when compared to those receiving the 3 + 0 vaccination schedule. Thus, when wanting to reduce the number of doses administered, a 2 + 1 schedule, with the third dose administered as a booster dose around 9 months of age, may be preferred over a 3 + 0 schedule in which all 3 doses are administered by approximately 14 weeks of age.

Trademark statement

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Data sharing statement

Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

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Authors' contributions

JPY, LS, MM, NF and SAM designed the study. AK, CC, LdG, LJ, NvN and SAM acquired the data. CC, DB, JPY, JRG, LdG, LJ, LS, MM, NF, NvN and SAM analysed the data. AK, CC, DB, JPY, LdG, LJ, LS, MM, NvN, SAM and SS contributed to the conduct of the study. All authors participated in the interpretation of the data. All reviewed and revised the manuscript, and approved the final manuscript as submitted.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: DB, JPY, JRG, LS, NF and SS are employees of the GSK group of companies and MM was an employee of the GSK group of companies. DB, JPY, JRG, LS, MM and SS own shares of the GSK group of companies. SAM's institution received grants from the Bill & Melinda Gates Foundation, the GSK group of companies, Novartis and Minervax and personal consulting fees for advisory boards and/or speaker's bureaus from the GSK group of companies, Medimmune, Pfizer and Sanofi Pasteur. All other authors declare no conflict of interest.

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All authors attest they meet the ICMJE criteria for authorship.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.01.062.

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